ARAUCARIAN SOURCE OF FOSSILIFEROUS BURMESE AMBER: SPECTROSCOPIC AND ANATOMICAL EVIDENCE

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ABSTRACT

Recent fossil discoveries show that Burmese amber is one of the most significant amber sites from the Early Cretaceous. We have used both nuclear magnetic resonance (NMR) and anatomical analyses to determine the plant source of amber taken from the Noije Bum 2001 Summit Site in the Hukawng Valley, Myanmar. All spectra were identified as belonging to Group A, which on the basis of a previous analysis of New Zealand amber and copal, is related to members of the Araucariaceae, especially *Agathis*. Bi- to multiseriate, angular, alternate, contiguous 5-6-sided intertracheal pitting on the fossil wood is typical of araucarioid pitting and only occurs in wood of extinct or extant members of the Araucariaceae. The amber from this mine site is considered to be derived from araucarioid (especialy *Agathis*) trees in the Araucariaceae.

Key Words: Burma, fossilized resin, tracheid pits, Nuclear magnetic resonance spectroscopy

RÉSUMÉ

De récentes découvertes chez les fossiles montrent que l'ambre birman est l'une des plus importantes sources de Crétacé ancien. Nous avons utilisé à la fois la résonnance magnétique nucléaire (NMR) et les analyses anatomiques pour déterminer la source botanique de l'ambre trouvé à Noije Bum 2001 Summit Site dans la vallée de Hukawng, Myanmar. Tous les spectres ont été identifiés comme appartenant au groupe A, qui sur la base d'analyses antérieures sur l'ambre et le copal de Nouvelle-Zélande est lié à des membres des Araucariacées, spécialement *Agathis*. Des trous trachéaux à 5-6 côtés, bi- à multisériés, angulaires, alternés, contigus, sur le bois fossile sont typiques des alvéoles des Araucarias ou d'araucarioides et se rencontrent dans le bois des membres des Araucariaceae. L'ambre de ce site minier est donc considéré comme venant *d'Agathis*, un genre actuel d'Araucariaceae."

INTRODUCTION

Burmese amber was traded with China as early as AD 100, but it was not until 1896 that fossils were reported in these deposits. In 1999, a new amber site was discovered in the Hukawng Valley in Myanmar (Poinar et al. 2005), and palynomorphs from this site were assigned to the Upper Albian of the Early Cretaceous (97–110 million years ago) (Cruickshank & Ko 2003). The same deposits have yielded the oldest bee (Poinar & Dancroft 2006), the oldest angiosperm flowers in amber (Poinar & Chambers 2005; Poinar 2004) and the first fossil evidence of vector borne diseases (Poinar & Poinar 2004), making it one of the most significant Cretaceous amber deposits in the world. Due to the scientific importance of this amber, we have used both spectroscopic and anatomical analyses to determine the plant source.

Clues to the determination of source trees of amber deposits can be provided by plant macrofossils or microfossils found in the amber or in sedimentary beds containing the amber and by spectroscopic analysis of the amber. A combination of these methods can then be used to describe source trees, as was done with *Agathis levantensis*, the araucarian tree responsible for the production of Middle East amber (Poinar & Milki 2001). Up to the present, the only spectroscopic evidence for the tree source of Burmese amber has been the nuclear magnetic resonance (NMR) analysis of three samples of Burmese amber (Lambert & Poinar 2002). The results of these analyses were conflicting with one sample unassignable (Lambert & Frye 1982), one related to the family Dipterocarpaceae (Lambert et al. 1999a) and the third related to the Araucariaceae (Lambert & Wu, unpublished research, 2002). Therefore, further analysis was undertaken with additional Burmese amber samples collected from this new site that was first mined in 2001. Tracheid fibers in one of the amber samples from this new site are characterized and used to provide anatomical evidence of the plant source.

MATERIALS AND METHODS

The amber samples analyzed in this study were collected from lignitic seams in sandstone-limestone deposits in the Hukawng Valley in Myanmar. The mine site was located on the slope of the Noije Bum hill about a mile (1.5 km) SSW of the old Khanjamaw mine site and southwest of Maingkwan (26°20'N, 96°36'E). Apparently this site had never been mined previously (Chhibber 1934) (Doug Cruickshank, pers. comm., December 20, 2006), and we refer to it as the "Noije Bum 2001 Summit Site."

Wood Fiber Analysis

A square piece of amber measuring approximately 23 mm on all sides and 6 mm thick contained numerous strips of wood fibers. This piece of amber was cut with a diamond saw along the flat side, leaving two narrow pieces of amber, each approximately 3 mm in thickness. The surfaces of these were polished and the wood fibers examined with a Nikon Optiphot optical microscope (with magnifications up to 800×). The amber pieces containing the wood fibers are deposited in the Poinar amber collection (accession# B-P-16) maintained at Oregon State University.

Spectroscopic Studies

Solid-state nuclear magnetic resonance spectroscopy with cross polarization and magic angle spinning (CP/MAS) was used to characterize the amber samples (Lambert et al. 1996). Both normal decoupling and interrupted decoupling modes were used to examine the solid-state ^{13}C NMR spectra.

Solid state ^{13}C NMR data were recorded on a 400 MHz Varian NMR System. The Direct Drive console had a clean rf architecture, a powerful digital receiver, and utilized advanced phase, amplitude modulation. The system had a 5 mm T3 PENCIL probe. The magic angle spinning rate was set to 5000 Hz. The cross polarization pulse sequence called tancpx was used for normal proton decoupling. For interrupted decoupling, the pulse sequence tancpxidref was used, in which a 50 µs delay was applied in the ^{1}H channel directly before the 180° pulse in the ^{13}C channel. A typical parameter set was as follows: spectrum frequency 100.544 MHz, spectral width 50 kHz, pulse width 3.4 µs for the 90° pulse for both ^{1}H and ^{13}C, delay time 5 µs, contact time 2 ms, acquisition time 20.5 ms, and scan number 256. Spectra were referenced to an external adamantane peak at δ 38.3 and were converted to tetramethylsilane at δ 0.0. Data were collected and processed with software VnmrJ 2.1B.

This procedure was performed on 5 separate samples (Nos.276, 375, 376, 422, 441) from the Noije Bum 2001 Summit Site. Four of the samples were clear (Nos. 276, 375, 422, 441) and one was opaque (376).

RESULTS

Wood Fiber Analysis

The tracheid surfaces contained 2–3 rows (2–3 seriate) of alternately arranged contiguous, angular, 5–6 sided tracheid pits (Fig. 1). Most of the bordered tracheid pits were hexagonal with diameters ranging from 11 to14 µm. None of these biseriate and triseriate pits possessed thickenings, such as crassulae or bars of Sanio between them. The polygonal pit cavities, which did not extend beyond the pit borders, varied from round to 5–6 sided (Fig. 1C). Resin globules occurred within some of the tracheids (Fig. 1C).

Spectroscopic Analysis

In all samples, the spectra with normal decoupling have the largest peak at δ 38 and a second, smaller peak at δ 18–20, with a broad grouping in the unsaturated region. With interrupted decoupling, the largest peak again was at δ 38, with nothing in the unsaturated region. All spectra were identified as belonging to Group

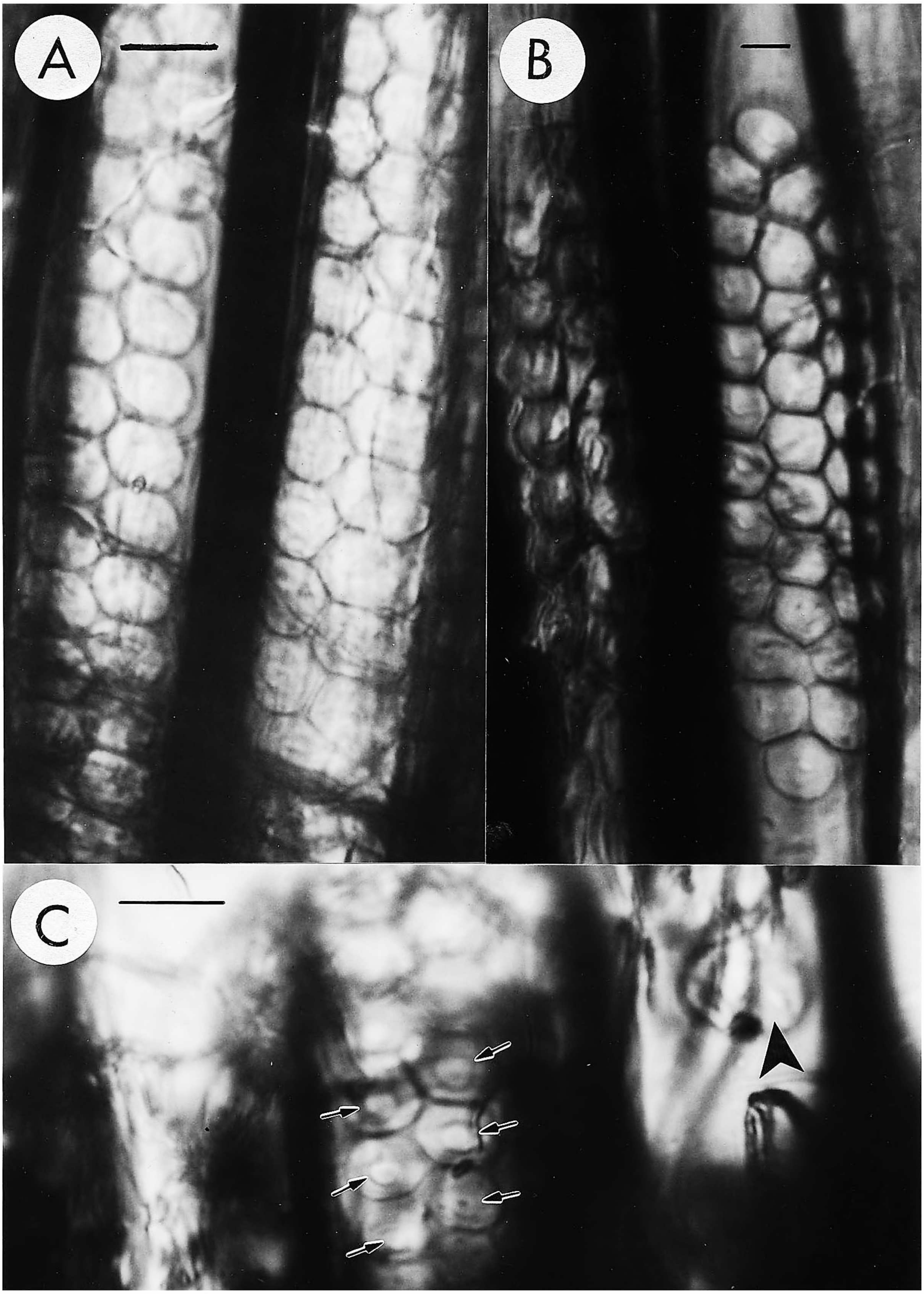

Fig. 1. A. Bi- and tri-seriate tracheid pits on a wood fragment in Burmese amber collected from the Noije Bum 2001 Summit Site. Scale bar = 14 µm. **B.** Contiguous, alternate, 5–6 sided pits commonly referred to as araucarioid pitting on a wood fragment in Burmese amber. Scale bar = 8µm. **C.** Pit cavities (arrows) on a tracheid fiber. Note excreted resin globule (arrowhead). Scale bar = 15µm.

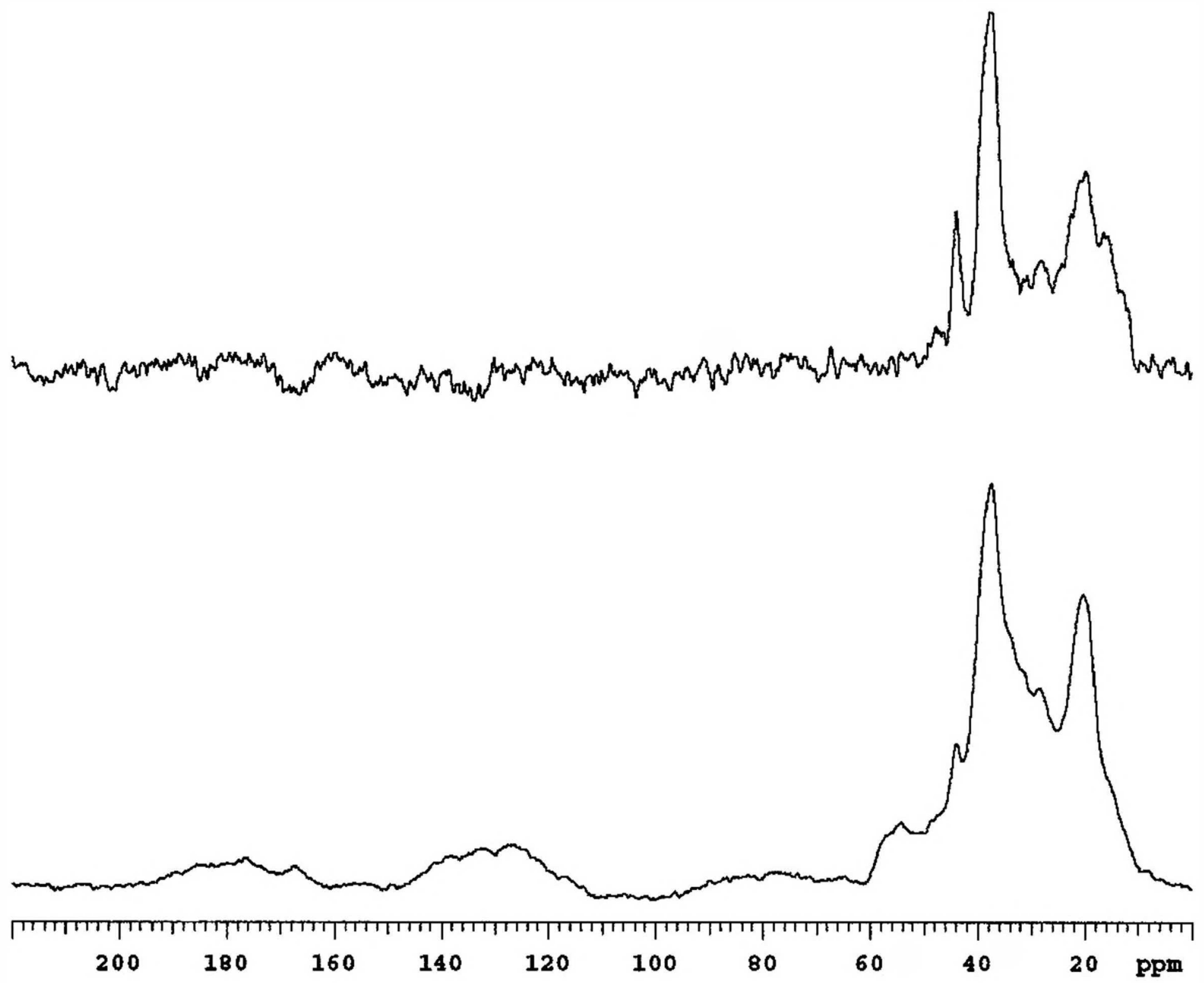

Fig. 2. ^{13}**C NMR spectra of Burmese amber sample 376 collected from the Noije Bum 2001 Summit Site with normal decoupling (lower) and interrupted decoupling (upper)(x axis indicates the unit δ in ppm).**

A (Fig. 2), which on the basis of a New Zealand series reported earlier (Lambert et al. 1993), is related to members of the Araucariaceae, especially *Agathis*, a genus commonly known as kauri pines now restricted to the Southern Hemisphere.

DISCUSSION

The arrangement of intertracheary pits and their cavities can be used to characterize familes and genera of conifers (Core et al. 1979; Patel 1968). Bi- to multiseriate, angular, alternate, contiguous, 5–6 sided longitudinal tracheid pits as seen on the radial walls of the tracheids, as found here, are commonly referred to as araucarioid pitting and only occur in wood of members of the Araucariaceae (Patel 1968; Tidwell 1998). Diameters of tracheid pits in members of the Araucariaceae vary from 12 to 16 µm (Core et al. 1979; Tidwall 1998) which is within range of the pits in the Burmese amber wood samples (11–14 µm). The presence of resin globules or plugs, as found in the fossilized wood, is characteristic of araucaroid tracheids (Patel 1968).

There are currently three extant genera in the family Araucariaceae: *Agathis*, *Aruacaria* and *Wollemia*. While the wood structure of *Agathis* and *Araucaria* is similar, (Langenheim 1995), recent resin of these two genera often can be distinguished by NMR analyses (Lambert & Poinar 2002), primarily because most *Araucaria* species produce gum resins (Lambert et al. 2005). In addition, resin deposits from *Araucaria* trees are small and do not polymerize and fossilize, as does *Agathis* (Langenheim 1995). While the NMR spectra of *Wollemia* and *Agathis* are similar (Lambert et al. 1999b), there is no evidence that *Wollemia* produces large deposits of resin that polymerize and form amber deposits.

Under normal decoupling conditions in NMR analyses, signals are obtained from all carbons present in the sample. With interrupted decoupling, signals are selected for quaternary carbons and carbons that are moving rapidly in the solid while other signals are edited out. These two spectral modes served as fingerprints to analyze and identify the Noije Bum 2001 Summit site amber samples as belonging to the worldwide Group A, which is most similar to members of the genus *Agathis* in the family Araucariaceae. Determination of this taxonomic grouping was based on comparisons with previously obtained NMR spectra of *Agathis* resin, copal and amber from New Zealand (Lambert et al. 1993). It has been found, however, that the ^{13}C fingerprint of modern resins (as opposed to gum resins) from the family Araucariaceae are indistinguishable from that of modern resins from the family Cupressaceae (Lambert et al. 2005). The presence of araucarioid wood fibers in the amber from the Noije Bum 2001 Summit site is collaborative evidence that the NMR spectra reported here are from a member of the Araucariaceae.

A spectroscopic comparison of fossil resins of the Cupressaceae with those of the Araucariaceae has not been made since it is difficult to obtain samples of fossilized resin associated with present day members of the Cupressaceae. There apparently are no localities that contain semi-fossilized or fossilized resin deposits of species of the Cupressaceae in soil surrounding the trees, swamps containing the stumps of former forests or in coal deposits, as occurs in New Zealand with *Agathis* (Poinar 1991; Lambert et al. 1993).

While some fragments of amber have been attributed to members of the Curpessaceae, (which now includes genera of the former family Taxodiaceae), there is no evidence that extant Cupressaceae produce copious amounts of resin under normal growth conditions. Members of this family only have a limited capacity to produce trunk resins (Langenheim 1995), the source of the majority of fossiliferous amber and then only when they experience severe traumatic stress. This is in contrast to species of *Agathis*, that produce not only copious amounts of trunk resins, but also were the source of fossilized resins (copal and amber) extending over thousands and millions of years and under a wide range of stratigraphic conditions (Halkett & Sale 1986; Poinar 1991; Lambert & Poinar 2002). While wood of the extinct conifer family Cheirolepidiaceae may also contain tracheids with araucaroid radial pitting, the absence of resin ducts and canals is a feature typical of these wood types (Axsmith & Jacobs 2005; Taylor & Taylor 1993), thus they could not have been the source of Burmese amber.

Determining araucarioid (very likely *Agathis*) trees as the source of Burmese amber is congruent with large amounts of pollen of the araucarian, *Araucaricites australis*, recovered in a palynological analysis of the sedimentary formation that contained the amber at the Noije Bum 2001 Summit Site (Davies 2001).

Commonly known as kauri or kauri pines, species of *Agathis* can become quite large and long-lived (from 500 to 1000 years) and have been compared with the Giant Sequoias of North America (Halkett & Sale 1986). By bulk alone, they would have formed a significant part of the Burmese amber forest. The climate of Burma was tropical- subtropical during the Early Cretaceous (Boucot et al. 2007), which is the preferred climate for extant *Agathis* species, all of which are now confined to the Southern Hemisphere (Poinar & Milki 2001).

Previous spectroscopic analyses of Burmese amber have been conducted with material from other sites. The first sample of Burmese amber tested (#7) was provided by Curt Beck and came from the Musée National d'Histoire Naturelle, Paris, (No. 101.604). The label stated it was collected from Mangotaimaw Hill, Myanmar. Its spectrum was broad and could not be assigned to any present group (Lambert & Frye 1982). In 1992, an analysis was performed on a piece of amber presented to the senior author by a commercial trading company located in Taiwan. This sample, (#154), which was dark brown, nearly opaque and lacked fossils, was represented as coming from the Hukong Valley in Burma. It produced a spectrum typical of the worldwide Group B, which is related to the extant angiosperm family Dipterocarpaceae (Lambert et al. 1999a; Lambert & Poinar 2002). In 2002, a sample of Burmese amber (#276) collected from the Noije Bum 2001 Summit Site, where samples were also taken by Cruickshank & Ko (2003) for their dating studies, showed a definite placement in the worldwide Group A, similar to the samples presented here. This result was simply cited in publications as Lambert & Wu, unpublished research (2002).

The present study shows that the amber from the Noije Bum 2001 Summit Site is produced from an araucarioid, quite probably a member of the genus *Agathis*. If sample #154 did originate from Burma, it indicates that at least two separate plant families (Araucariaceae and Dipterocarpaceae) were sources of amber in Myanmar, quite possibly at two distinct geological periods.

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